Extraction of natural colourants from roots of *Morinda angustifolia* Roxb.—
Their identification and studies of dyeing characteristics on wool

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The colour components in the roots of the plant *Morinda angustifolia* Roxb. were isolated and their structures were elucidated based on chemical and spectroscopic investigations. Further, the dyeing behaviour of the colour component on wool in aqueous medium was also evaluated. Depending on concentrations of dye (1-5%) in the dye bath, the dye absorption on the fibre varied from 12.50-31.38%. The fastness properties of the dyed yarns were determined. Optical densities (OD) and colour strength (K/S) values of the dye solutions as well as the dyed yarns and also colour co-ordinates for dyed yarns were determined. Also, the dyeing characteristics of the colouring matter on wool were studied with and without the use of mordants and the properties of the dyed fibres were evaluated.

**Experimental Procedure**

**Raw materials**

Fresh roots of *Morinda angustifolia* (5 kg) were collected from a forest near Jorhat, India during winter season. The roots were finely washed with tap water and dried. The dried roots were cut into small pieces and then ground to fine powder.

Four-ply worsted yarn of Australian Merino Wool was used for dyeing experiments. Mordants such as 

- CuSO₄·5H₂O (LR, CDH), K₂Cr₂O₇ (LR, CDH), SnCl₂·2H₂O (LR, CDH) and Al(NH₃)₂(SO₄)·12H₂O (LR, Qualigens) were used.

Distilled water was used for extraction of the colour components and for preparation of all chemical solutions, while de-ionised water was used for dyeing purpose.

**Isolation of colour components from the roots by solvent extraction**

The air-dried powder of the roots of *Morinda angustifolia* (1 kg) was extracted with ethanol in a soxhlet apparatus for 10 h. The ethanol was then removed under reduced pressure to get a solid mass, which was then successively extracted with petroleum ether, benzene and methanol.
The petroleum ether extract on preparative TLC and subsequent column chromatography eluting with n-hexane, n-hexane:ethyl acetate at ratios 30:1, 25:1, 25:2, 10:2 yielded three fractions — F-1, F-2 and F-3. The benzene extract was then passed through a column of silica gel (60-120 mesh) and eluted with n-hexane: ethyl acetate at ratios 30:1, 20:1, 10:2, which yielded two more fractions — F-4 and F-5. The methanol extract gave another three fractions — F-5, F-6 and F-7 upon eluting with different ratios of n-hexane and ethyl acetate.

**Evaluation of the colour components by spectral analyses**

Infra red (IR) spectra of the above isolated compounds were recorded on a Perkin-Elmer spectrophotometer (Model 580B) using KBr disk technique, in the range 4000-200 cm⁻¹. The ultra violet/visible (UV/Vis) absorption spectra of compounds were recorded on a Shimadzu 1601 PC UV/Vis spectrophotometer using methanol in the range 200-800 nm. The nuclear magnetic resonance (NMR) spectra were recorded on a EM 360 L (60 MHz) NMR spectrophotometer using DMSO-d₆ in the range 0-10 δ with trimethylsilane as an internal standard. The mass spectra were recorded on a Finnigan-MAT (INCIOS-50) spectrophotometer. The melting point of the compounds were determined on Buechi B-540 melting point apparatus and were un-corrected. The elemental analyses were done in a Perkin-Elmer 2400 elemental analyzer.

**Aqueous extraction of colour components from roots for dyeing purpose**

The finely ground root materials (100 g) with 12% moisture were extracted with 500 mL distilled water for about 2 h at temperature 90°C. The extract was then concentrated under reduced pressure over a boiling water bath. The yield of the dried powder was found to be 15.2%.

**Optical density and colour strength measurement**

Dye solutions varied from 1-5% were prepared and definite amounts were taken in the dye-bath by maintaining the material to liquor (M:L) ratio at 1:10. The absorbance of the solution was recorded before and after dyeing of wool at 440 nm in each case. An average of 3 absorbance measurements at each concentration was recorded. The dye absorbance was calculated as given below:

\[
\text{Absorbance} = \frac{\text{Absorbance before dyeing} - \text{Absorbance after dyeing}}{\text{Absorbance before dyeing}} \times 100
\]

Further the colour strength (K/S) values of the dye solutions as well as the dyed samples were evaluated by light reflectance technique using a Shimadzu 1601 PC UV/Vis spectrophotometer. The K/S values were assessed using the Kubelka-Munk equation

\[
K/S = \frac{(1-R)^2}{2R}
\]

where, \( R \) is the observed reflectance, \( K \) is the absorption coefficient and \( S \) is the light scattering coefficient.

**Dyeing of wool**

The worsted yarn was first scoured as per the BIS method (IS:1349:1964)¹⁴. The scoured yarn was subjected to ethanol extraction in a soxhlet apparatus at the rate of six siphons/h for 3 h, rinsed with distilled water and finally dried at room temperature. This was done to ensure the removal of residual soap etc from the fibre.

The dyeing was carried out at temperature 97-98°C in a dye bath containing 3% dye at m:L ratio 1:10 for 45 min. Then, a 2% sodium chloride solution on the basis of material was added to the dye bath and the system was further kept at that temperature for 15 min. The dyed yarns were then washed and dried at room temperature.

**Method of mordanting**

Pre- and post-mordanting methods using 2% solutions of each of CuSO₄·5H₂O, K₂Cr₂O₇, SnCl₂·2H₂O and Al(NH₄)(SO₄)₁₂·12H₂O were employed at m:L ratio 1:10 and mordanting was carried out for 30 min at 97-98°C. The fibres were then washed and dried.

**Measurement of fastness properties**

Colour fastness tests to light, washing and crocking were carried out in a Fade-O-meter, Launder-O-meter and Crock-O-meter respectively as per the standard methods¹⁵.

**Hunter coordinates**

The Hunter coordinates \( L, a \) and \( b \) were calculated from the tristimulus values \( x, y, z \) using the following equations¹⁶ and were converted to CIELab coordinates.
$L = 10y^{1/2}$

$a = 17.5(1.02x - y)y^{1/2}$

$b = -(y - 0.84z)/y^{1/2}$

The higher values of $a$ and $b$ indicate brightness, which is more due to redness and yellowness respectively, and the negative values indicate greenness and blueness which are more towards the dull side. The lower the value of $L$, the greater is the depth.

**Results and Discussion**

**Characterization of the colour components**

The characteristics of the isolated compounds F1 - F7 were as given below:

**Compound F1:** White needles (25 mg); m.p 134-135°C; UV $\lambda_{max}$ (C2H5OH) nm: 280 nm; IR $\nu_{max}$ (KBr) cm$^{-1}$: 3570 (-OH), 1650; $^1$H NMR (CDCl3) $\delta$: 1.39 (3H, d, J=6.5 Hz, 29 Me), 2.75 (1H, m, 6H), 4.9 (1H, m, 28H); Anal. for C$_{15}$H$_{10}$O$_{5}$: Found C=83.92%, H=12.12% (Calc. C=83.98% and H=12.15%); MS; m/z 414 [M+], 399, 396, 381, 303, 273. [$\alpha$]$_D^{25}$ -36° in CHCl$_3$. These results showed that the compound is β-Sitosterol.$^{17}$

**Compound F2:** Orange-yellow needles (1 gm); m.p 220-221°C; UV $\lambda_{max}$ (MeOH) nm: 225(4.52), 256 (4.31), 278 (4.03), 286 (4.02), 430 (4.01); IR $\nu_{max}$ (KBr) cm$^{-1}$: 3400 (-OH), 1670 (w, C=O, unchelated), 1630 (s, C=O, chelated), 1570 (aromatic C=C); $^1$H NMR (DMSO-d$_6$) $\delta$: 7.20 (1H, d, H-2), 7.42 (1H, d, H-3), 7.16 (1H, d, H-4), 7.81 (1H, d, H-5), 7.79 (1H, d, H-6), 7.68 (1H, s, H-7), 3.68 (s, -CH$_3$); MS; m/z 270 [M$^+$. Anal. for C$_{15}$H$_{10}$O$_{5}$: Found C=66.5%, H=3.8% (Calc. C=66.6% and H=3.7%).

From the above results, the compound may be determined to be Aloe-emodin.$^{18}$

**Compound F3:** Yellow-brown needles (50 mg); m.p 252°C; UV $\lambda_{max}$ (MeOH) nm: 254, 269, 290, 438; IR $\nu_{max}$ (KBr) cm$^{-1}$: 3390, 1675, 1631; $^1$H NMR (DMSO-d$_6$) $\delta$: 7.03 (1H, d, H-2), 7.34 (1H, d, H-3), 7.15 (1H, d, H-4), 6.81 (1H, d, H-5), 3.11 (Ab -CH$_3$, s); MS; m/z 270 [M$^+$, 100%]. Anal. for C$_{15}$H$_{10}$O$_{5}$: Found C=66.57%, H=3.79% (Calc. C=66.67% and H=3.73%). Here again, the compound is determined as Emodin.$^9$

**Compound F4:** White shiny crystals (50 mg); m.p 283°C; IR $\nu_{max}$ (KBr) cm$^{-1}$: 3400-3500 (-OH), 1680 (-COOH), 1640 (unsaturation); 2920, 1440, 1380; $^1$H NMR $\delta$ (CDCl$_3$): 0.65 (3H, s, -CH$_3$), 0.67 (3H, s, -CH$_3$), 0.78 (3H, s, -CH$_3$), 1.26 (6H, s, 2 -CH$_3$), 1.06 (6H, d, J=6 Hz, s-CH$_3$), 5.27 (1H, m, vinyllic H), 4.41 (1H, m, -CHOH); MS; m/z 456, 248 (base peak); Anal. for C$_{15}$H$_{18}$O$_{5}$: Found C=78.94%, H=10.62% (Calc. C=78.89% and H=10.59%); [$\alpha$]$_D^{21}$ +66° in EtOH and KOH.

These results showed that the compound is Ursolic acid.$^{20}$

**Compound F5:** Orange-red needles (2 gm); m.p 280°C; UV $\lambda_{max}$ (MeOH) nm: 446, 299, 291, 265.5 and 232; IR $\nu_{max}$ cm$^{-1}$ (KBr): 3400 (-OH), 2890 and 1400 cm$^{-1}$ (-CH$_3$), 1670 (unchelated C=O), 1600 cm$^{-1}$ (chelated carbonyl group). $^1$H NMR (DMSO-d$_6$) $\delta$: 2.29 (3H, s, benzyl Me), 7.49 (1H, d, J=8.0 Hz, H-3), 7.73 (1H, d, J=8.0 Hz, H-4), 7.17 (1H, d, J=9.0 Hz, H-7), 8.07 (1H, d, J=9.0 Hz, H-8); Anal. for C$_{15}$H$_{18}$O$_{5}$: Found C=66.59%, H=3.75% (Calc. C=66.67% and H=3.73%). MS; m/z 270 [M$^+$, 100%]. This was found to be in perfect agreement with that of the compound Morindonin.$^{21}$

**Compound F6:** Yellow needles (30 mg); m.p > 300°C; UV $\lambda_{max}$ (MeOH) nm: 227, 265, 437; IR $\nu_{max}$ (KBr) cm$^{-1}$: 3300 (-OH), 2990, 2980, 1698 (unchelated C=O), 1630 (chelated C=O) 1454, 1268; $^1$H NMR $\delta$ (DMSO-d$_6$): 8.02 (1H, d, J=1.5 Hz, H-8), 7.82 (1H, d, J=1.5 Hz, H-7), 7.71 (1H, d, J=1.5 Hz, J=7.5 Hz, H-5), 7.39 (1H, d, J=7.5 Hz, H-6), 7.57 (1H, d, J=7.5 Hz, H-1); MS; m/z 284 (M$^+$, 100%); Anal. for C$_{15}$H$_{18}$O$_{5}$: Found C=63.40%, H=2.85% (Calc. C=63.39% and H=2.84%).

Here again, the compound is determined as Rhein.$^{22}$

**Compound F7:** Orange-yellow needles (50 mg); m.p 244°C; UV $\lambda_{max}$ (MeOH) nm: 232, 262, 287, 298 and 440; IR $\nu_{max}$ (KBr) cm$^{-1}$: 3480 (-OH), 1675 (unchelated C=O), 1625 (chelated C=O); $^1$H NMR $\delta$ (DMSO-d$_6$): 7.39 (1H, d, J=8 Hz, H-3), 7.72 (1H, d, J=8 Hz, H-4), 7.16 (1H, d, J=9 Hz, H-7), 7.96 (1H, d, J=9 Hz, H-8), 2.32 (3H, s, Ar-CH$_3$), 3.5-4.9 (12 H, sugar protons); MS; m/z 564 (M$^+$, 100%); Anal. for C$_{22}$H$_{28}$O$_{14}$: Found C=55.40%, H=4.97% (Calc. C=55.32% and H=4.99%). [$\alpha$]$_D^{21}$ -82.6° in dioxane. These results showed that the compound is Morindonin.$^{23}$

The structures of the above isolated compounds are given in Fig. 1.
As evident from above, except β-sitosterol which is a steroid and ursolic acid, a triterpenoid, the major portion of the chemical compounds present in the extracted colouring matter are anthraquinone based compounds.

**Effect of concentration of dye on optical density, absorption and colour strength**

As is evident from Table 1, the absorption of dye (%) on fibre increased with an increase in concentration of dye in the dye-bath and reached maximum at 3%. Though the K/S values went on increasing with an increase in dye concentration, the maximum absorption (31.58%) was observed at 3%. At this optimum concentration of dye, desired fastness properties on fibre were also obtained (Table 2).

<table>
<thead>
<tr>
<th>Dye cone (%)</th>
<th>Optical density</th>
<th>Absorption (%)</th>
<th>K/S</th>
</tr>
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<tbody>
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</tr>
<tr>
<td>1</td>
<td>0.16</td>
<td>0.14</td>
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<td>3</td>
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<td>0.20</td>
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<tr>
<td>5</td>
<td>0.22</td>
<td>0.17</td>
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</table>

Table 2—Dyeing properties of colour components extracted from roots of *Moringa angustifolia* Roxb

<table>
<thead>
<tr>
<th>Mordants</th>
<th>Mordanting technique</th>
<th>Light fastness</th>
<th>Crocking fastness</th>
<th>Wash fastness</th>
<th>Shade on fabric</th>
</tr>
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<tbody>
<tr>
<td>Nil</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>Golden brown</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>I</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>Grey</td>
</tr>
<tr>
<td>K₂Cr₂O₇</td>
<td>I</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>Dark brown</td>
</tr>
<tr>
<td>SnCl₂·2H₂O</td>
<td>I</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>Al(NH₄)₂(SO₄)₆·12H₂O</td>
<td>I</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>Yellowish brown</td>
</tr>
</tbody>
</table>

1—pre-mordanting. II—post-mordanting.
1—very poor, 2—poor, 3—fair, 4—very fair, 5—good, 6—very good.
Effection
Moreover, desired fastness properties on ever beyond 2% concentration of mor­
dant, the increase in dyeing when mordant­
e or metal ions was found to follow the sequence—
Cu(II)~Cr(VI)~Sn(II)~Al(III). The brighter shades were obtained when mordanted with
Cr(VI) and Cu(II). This might be due to the maximum absorption and easy formation of metal-complexes
with the fiber 24,25. Fair to good wash and crocking fastness properties were obtained when mordanted with
CuSO4·5H2O and K2Cr2O7,26 while lighter shades with fair to good wash and crocking fastness properties
were obtained with SnCl2·2H2O and Al(NH4)2(SO4)2·12H2O.27 Further, it was also evident from Table 2 that better fastness properties were obtained when using post-mordanting technique.

Evaluation of colour coordinates of dyed yarns
The results on colour coordinates of dyed samples showed that the values obtained for L when pre­
mordanted with all the four mordants were varied from 39.81-65.27, while the values obtained for post-
mordanted fibre varied from 38.18-63.66. So also the values obtained for a in both pre-and post-mordanted
fibres varied from 10.19-31.98 and 11.43-25.13 and that for b from 9.13-75.14 and 28.78-76.13 respectively. For unmordanted dyed fibres, the values for L, a and b were 56.16, 24.18 and 63.84 respectively. It was observed that all the colour coordinates were positive with respect to brightness L, red-green a,
yellow-blue b and therefore, all of them lie in the yellow-red quadrant of the colour space diagram. The maximum brightness was observed in the samples dyed and post-mordanted with SnCl2·2H2O (L 65.27). The lowest brightness was observed in the samples dyed and post-mordanted with CuSO4·5H2O (L 38.18).

Conclusion
From the above study, it might be concluded that the colour components isolated from the roots of
Morinda angustifolia Roxb. contained anthraquinone moiety in their molecules. Wool yarns could be dyed to a wide range of shades with this colour component alone or by using different mordants. Thus, the dye so extracted may be an alternative to synthetic dye for dyeing of wool.

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References